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The effect of ambient temperature on recovery of surgical stress in Sprague-Dawley rats

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The Effect of Ambient Temperature on Recovery of Surgical Stress in Sprague-Dawley Rats

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Table of Contents

Preface I

List of Abbreviations

- Sprague-Dawley (SD)
- Ambient temperature (Tamb)
- Core temperature (Tc)
- Non-shivering thermogenesis (NST)
- Selected ambient temperature (STa)
- Thermal neutral zone (TNZ)
- Lower critical temperature (LCT)
- Upper critical temperature (UCT)

Preface II

Tables

Figures

Abstract

Laboratory animals are housed at ambient temperatures ranging from 20°C – 26°C per recommended guidelines. Rats in particular typically prefer ambient temperatures (Tamb) of ~27°C (Brown et al, 2011). When rats undergo surgical instrumentation for experimental use, they often recover at normal room temperature (~21°C). While this is comfortable to those maintaining them, it may lead to a cold thermal stress for the rats. It is hypothesized that housing rats at ambient temperatures away from their preferred Tamb could lead to a thermal stress, which adversely affects surgical recovery. To address this, rats (220g-350g) were surgically instrumented with a radiotelemetry probe (DSI), which allows non-invasive measurement of core temperature (Tc). A cannula (21ga) was also inserted into the brainstem to allow microinjection of drugs as part of a separate project. For at least 1 week post-surgery, the rats were housed at either 21°C, 24°C, 27°C (control), 30°C, or 33°C Tamb while Tc, food and water intake, and body weight changes were measured. Rats housed at 21°C and 24°C struggled to recover a normal Tc initially, which delayed the onset of normal circadian cycling in the 21°C group. Warmer Tamb (30°C & 33°C) did not alter recovery of circadian Tc rhythms. Rats held at 21°C steadily increased food intake, while those at 33°C consumed less as the recovery period continued, suggesting that colder rats needed more caloric intake to thermoregulate, while those at warmer Tamb did not. Rats in the 30°C and 33°C groups consumed more water after post-surgical Day 3 compared to the other groups. This was due to more evaporative water loss at the higher Tamb versus the rats held at lower Tamb. The return of body weight to pre-surgical levels in rats housed at 33°C was delayed due to heat stress. The control group (27°C) returned to pre-surgical weight

earlier than the other groups. These data suggest that rats maintained at 27°C recovered from surgical stress more readily. These rats returned to pre-surgical body weight more quickly and demonstrated a normal thermoregulatory circadian rhythm earlier than the cold (21°C) rats. The rats housed at 33°C were exposed to a heat stress, which affected weight gain and surgical recovery initially but did not affect circadian cycling during recovery. Rats housed at 21°C were cold stressed, which affected weight gain and thermoregulatory recovery. It is suggested that rats be maintained at their preferred Tamb of 27 °C during the week following surgery to minimize thermal stress and thereby facilitate recovery. This reduction in thermal stress would facilitate the return to a normal physiologic state and consequently enables more reliable data collection from these animals.

Introduction

Statement of the Problem

Many researchers use lab animals to explore physiologic responses to various stimuli. Using animals requires housing them within specific environmental parameters to ensure the health of the animals. An important aspect of these housing conditions is maintenance of appropriate ambient temperature (Tamb). This is especially important in animals that are exposed to other stressors such as surgical instrumentation in preparation for experimentation. It is proposed that current Tamb guidelines for rodents, such as Sprague Dawley (SD) rats, may allow for thermal stress, which affects the animal's overall physiology and ability to recover from surgery. The current guidelines of the NIH Guide for the Care and Use of Laboratory Animals suggest a Tamb range between 20°C to 26°C (68°F to 79°F), which is comfortable for researchers and staff, but below the preferred Tamb of ~27°C for SD rats (Brown et al., 2011). Although housing design, bedding for nesting material, and grouping animals in cages together are used as resources to minimize thermal stress, animals are still at great risk for thermal stress despite adherence to housing guidelines (Institute for Laboratory Animal Research, 2011). Surgical recovery requires special care because of the stress from surgery. The current guidelines suggest warming animals post-surgery, but the degree and duration are not established. Without a proper establishment of Tamb guidelines following surgery, the physiology of the animal may be abnormal, which may result in a stressed rat being used in experimentation. This could yield invalid data and confound interpretation. It is essential to refine such housing guidelines not only improve the care and use of laboratory animals, but also to validate data gathered from survival surgery experiments. The goal of this study is to determine the optimal ambient temperature range for housing rats during recovery from surgical stress.

Background

The regulations set forth by the NIH Guide for the Care and Use of Laboratory Animals aim to ensure that lab animals are housed in an environment that minimizes stress. Thermal stress can affect the overall health of the rats as well as recovery from experimentation and surgical instrumentation. Current guidelines suggest housing rats between 20°C to 26°C (68°F to 79°F) (Institute for Laboratory Animal Research, 2011). This suggested range is based on thermoregulatory behaviors noted during normal housing. Unfortunately, for many species such as rodents, these recommendations may not be appropriate as rectal probes were used to measure core temperature (Tc) in defining a basis for the guidelines. These probes likely stressed the animal and therefore potentially confounded data collected. Furthermore, current research suggests that the preferred ambient temperature of these rats is 27°C (Gonder & Laber 2007). This is higher than the current housing guidelines, suggesting these guidelines may lead to cold stressed rats (Brown et al., 2011).

Thermoregulation

Thermoregulation is the body's attempt to regulate core temperature (Tc) within normal limits regardless of environment temperature. SD rats maintain a Tc between 36.5°C -38.5°C which fluctuates around a set point of ~37.5°C (Gordon, 1993). Body temperature regulation relies on the nervous system's ability to sense and integrate thermal information from the external environment and deep within the body core. The hypothalamus, an area of the brain responsible for much of the homeostatic regulation of the body, contains the preoptic area (POA). This area is thought to integrate information about local brain temperature and other body temperatures and to control the level of output of thermoregulatory responses (Bicego et al., 2007). The POA compares the body's current Tc based on information sent from afferent neurons, to the thermoregulatory set point, and coordinates appropriate effector responses if corrections are needed. These various efferent mechanisms, or thermo-effectors, are used to either cool down or heat up the body, depending on what information was perceived.

Thermo-effectors modulate the Tc through altering heat production and heat loss mechanisms, which allow Tc to be maintained at the set point. One important thermo-effector in rodents is brown adipose tissue. It is responsible for non-shivering thermogenesis (NST), a mechanism used by rats to generate heat by making ATP production inefficient (Cannon & Nedergaard, 2011). When the Tc decreases below the set point, a large amount of lipids and glucose are combusted in brown adipose tissue to generate heat. This allows more heat to be produced than normal as a byproduct of metabolism. The rats will use the brown adipose tissue to generate energy for heat without shivering. Blood leaving brown adipose tissue is nearly depleted of oxygen after stimulating the tissue in a cold environment (Cannon & Nedergaard, 2011). After about nine months postnatal, humans lose their brown adipose tissue as their surface area to volume ratio decreases. This is important in rats because their surface area to mass ratio remains high into adulthood and they do not shiver. They instead depend on brown adipose tissue for NST.

Rats can rely on peripheral vasomotor tone to maintain their Tc as well. The tail of rodents in particular is crucial for thermoregulation using vasoconstriction or vasodilation to regulate heat loss. The reasons that the tail is ideal for heat release are 1) its lack of fur, 2) its vascularity, and 3) its high surface area to volume ratio. Warmer environments cause increases of blood flow through the tail from vasodilation, which radiates heat. In contrast, a colder environment will cause tail vasculature to vasoconstrict, decreasing blood flow and minimizing heat loss (Gordon, 1993).

When the rat's Tc rises above or below the set point, behavioral mechanisms of thermoregulation are activated. Rats will seek cooler environments and adopt a sprawled posture to lose heat. In addition, some behaviors are integrated such as grooming while also secreting saliva, which is then spread on the skin by the rat. This facilitates an evaporative heat loss on the skin. This is analogous to humans sweating in a warm environment. Rats do not sweat so this behavioral response in rodents facilitates heat loss. In a colder environment, rats will burrow into their bedding or stay grouped together to limit heat loss (Brown et al., 2011; Gordon, 1993). These behavioral thermo-effector mechanisms allow Tc to be maintained within normal limits of a set point despite significant changes in Tamb.

The circadian cycle also affects thermoregulation. Rats are nocturnal so their motor activity increases at night. This generates heat, which increases Tc. Rats will typically choose a slightly cooler environment at night to offset this heat production (Brown et al., 2011). When at rest during the daytime, Tc decreases with the lack of motion-generated heat and the selected ambient temperature (STa) increases in response. STa is the preferred temperature the rat will choose depending on its thermal needs. If given the option to choose Tamb, rats will behaviorally choose a STa that is closer to their preferred Tamb (Brown et al., 2011; Gordon, 1993). Generally about an hour before nightfall, rats will increase their motor activity (MA) and Tc in anticipation of the dark photoperiod. The inverse is true in anticipation of the light photoperiod in the morning. In this study, the photoperiod change occurs at 07:00 and 19:00. Therefore the time periods between 10:00-16:00 and 22:00-04:00 show a relatively stable Tc and MA. Careful examination of circadian thermoregulatory responses after surgery will be a key aspect of this project.

Thermal Neutral Zone

The thermal neutral zone (TNZ) is the range of ambient temperatures that allow a minimal need for energy expenditure while maintaining Tc (Gonder & Laber, 2007). The lower critical temperature (LCT) and upper critical temperature (UCT) are the minimum and maximum ranges of the TNZ before the rats use thermo-effector pathways to generate or lose heat in order to maintain Tc at the set point. In Sprague-Dawley rats, the preferred ambient temperature is 27°C, which is between the LCT of 26°C and the UCT of 30°C (Brown et al., 2011). The current housing guidelines (20°C-26°C) allow housing at Tamb below the LCT and preferred Tamb, therefore outside of the TNZ (26°C-30°C).

Thermal Stress

Housing rats at ambient temperatures outside of their TNZ, and therefore away from their preferred Tamb, likely elicits thermal stress responses. Such changes could be considered additional experimental variables, and should be taken into consideration regarding data collection and interpretation. In an experiment conducted by Zylan and Carlisle (1992), Tamb was varied to observe its effect on brown adipose tissue in rats and the amount of oxygen consumed during non-shivering thermogenesis. During baseline experimentation, oxygen consumption increased as Tamb decreased, indicating that 30 minutes of cold exposure was sufficient enough to induce a significant metabolic response. As stated above, such thermal stress might confound data collection and interpretation based on significant increase in energy expenditure and subsequent change the normal physiological state of the rodent. Rats prefer an ambient temperature of 27 °C (Brown et al., 2011). This varies slightly with circadian rhythm in which the rats prefer slightly cooler temperatures at night and slightly warmer ones during the day. During active periods, rats will choose Tamb below LCT; however, they prefer temperatures above their LCT for maintenance and resting, which is essential for everyday function (Institute for Laboratory Animal Research, 2011). Rats typically do not have the opportunity to choose their own STa in a normal vivarium and must rely on thermoregulatory processes to manage thermoregulation.

If an animal is housed at a Tamb below the LCT, then cold stress can chronically activate both thermo-effector pathways that limit heat loss and also chronically increase metabolism. An experiment conducted by David et al. (2013) found that chronic cold stress requires greater energy expenditure to manage core body temperature, likely by activation of brown adipose tissue and subsequent NST. This can cause the failure of rodent models to emulate human physiology. In the same experiment, with the vivarium temperature being lower than the TNZ, but within housing guidelines, energy expenditure of mice was increased significantly when compared with intermediate and heated temperatures. There was an associated shift in metabolism toward glucose utilization for energy production (David et al., 2013). The use of metabolism to maintain core temperature can be problematic during recovery from surgical stress because energy is being split between recovering and thermoregulation.

Warm stress occurs when the Tamb is above the UCT, activating thermo-effectors such as sweating in humans or grooming (saliva spreading on skin) in rodents to facilitate body heat loss to the environment. According to Leon et al. (2005), mice that show these thermoregulatory responses to heat stress are key biomarkers that may provide insight into heat stroke pathophysiology. Raising Tamb outside the TNZ is risky in that hyperthermia can lead to heat stroke and the eventual denaturing of vital proteins in the body. While a cold stress does not affect cellular function in the same ways, a warm stress does require activation of thermo-effectors to cool the core temperature to within normal limits. During surgical recovery, however, briefly increasing the Tamb is useful in helping the animal return to their normal Tc. To what degree and duration the Tamb should be raised is not yet known. Warming may be appropriate in the post-surgical period to facilitate recovery, but excessive and prolonged warming may prove equally stressful to the animal.

Ambient Temperature in Surgical Recovery

Ambient temperature plays a significant role during surgical recovery. When rats are given an anesthetic, they experience hypothermia, where the Tc will drop significantly unless external warming is significant. It is important to note, hypothermia during anesthesia from a lack of thermoregulatory control from the hypothalamus is the most common perioperative thermal disturbance in rodents (Diaz & Becker, 2010). Anesthetics impair the hypothalamus, thus thermoregulation as well, suggesting external warming to prevent hypothermia is needed. However, hypothermia can be beneficial during surgery. For example, in cardiopulmonary bypass, following traumatic brain injury, and many other clinical circumstances, hypothermia is used to lower the demand for oxygen, which allows aerobic metabolism to continue through greater periods of compromised oxygen supply. This thereby reduces the production of anaerobic byproducts such as superoxide radicals and lactate (Diaz & Becker, 2010). While hypothermia can be beneficial in surgery, once anesthesia wears off, the animal must spend metabolic energy to recover and maintain Tc. However, housing at Tamb below the TNZ for extended periods will force animals exposed to surgical stress to use metabolic resources to thermoregulate instead of using them for surgical recovery.

How to Address the Problem:

Current guidelines allow for housing of rats in an environment that may lead to a cold stress, which can result in stressed animals being used in experimentation and therefore potential collection of misleading data. The specifics of Tamb requirements during surgical recovery are unclear as the Guide briefly mentions raising the Tamb, but does not specify the degree or duration. If subjects are exposed to a significant surgical stress while being housed below their LCT, the combined surgical stress could adversely affect recovery. If housed above their UCT during recovery, the increased heat may help return to normal Tc faster, but also has the potential to eventually cause a heat stress. It is therefore essential to better define the housing guidelines to determine the appropriate Tamb during surgical recovery.

It is therefore hypothesized that *housing rats at ambient temperatures outside of their thermal neutral zone leads to a thermal stress, which adversely affects surgical recovery. This would be evidenced by alterations in food and water intake, delayed return of body weight to pre-surgical levels, and alterations in re-establishing normal levels, and circadian cycling of, core temperature. Rats housed at their preferred Tamb of ~27C are anticipated to be less stressed and recover more quickly than other* *experimental groups.*

Implications

Rats are used extensively for experimentation to explore physiologic variables in health and disease. Not only are animal models important for understanding physiology and pathologies, but also for testing hypotheses that cannot be done in human studies (Horvath, et al., 2015). Many times surgical instrumentation with devices that monitor physiologic variables are required. Recovery from surgical instrumentation to a minimally stressed state before experimentation begins is essential to the collection of quality data from these animals. The scope of the present study is to better define appropriate housing regulations that inform better living standards of rats, shorter recovery periods after surgical stress, and higher quality data collection from surgically instrumented animals. If data suggest current housing guidelines allow for thermal stress in animals, previously collected data from animals still recovering from surgery may need to be re-evaluated to ensure data is valid. Refinement of housing guidelines in regards to these important issues is essential to help facilitate animal recovery from stress, improve data quality, and thereby aid in the Reduction, Refinement, and Replacement (“Three R’s”) of laboratory animals in scientific research (Institute for Laboratory Animal Research, 2011).

Methods

The following experimentation used a previously approved surgical procedure involving implantation of a radiotelemetry probe into the abdominal cavity, which was used to non-invasively measure Tc (IACUC Protocol #A16-04). Also during surgery, a microinjection cannula was inserted into the brainstem to microinject drugs that alter neurotransmission as a part of a separate project.

Animals Used

Male and female SD rats weighing between 225 to 350g and between 7 to 10 weeks old were used in this study for numerous reasons. First, SD rats are one of the most commonly used strains in physiological and thermoregulatory research. Secondly, previous work that examined the preferred Tamb of rats used this strain (Brown et al., 2011). Using SD rats in this study would allow direct comparison of findings between studies. If the ideal Tamb for surgical recovery was the same as the unstressed rat's preferred temperature, it would suggest a narrow range of Tamb in which to house SD rats. If it differed, this would suggest the effect of surgical stress alters thermoregulation and therefore, rats experiencing this stress require special housing after surgery, which addresses their thermoregulatory needs. Finally, the normal thermoregulatory responses to various stimuli are well established in this readily available strain, which makes comparison of abnormal thermoregulatory behaviors and responses easier to identify in stressed rats.

Animal Housing

During the normal pre-surgical period, rats were housed in polycarbonate cages (50 x 26.8 x 36.4 cm) fitted with Hepa-filter cage tops and corncob bedding (Harlan Inc).

Figure 1: Cage Top Warmer. It rests atop the cage and below the HEPA filter top and circulates temperature controlled air to hold the rat's Tamb steady at the desired level.

Rats would remain at Tamb of 25 ± 1°C, 40 ± 5% relative humidity, with a 12:12 hr L:D cycle (lights on at 0700h). Laboratory rodent chow (Harlan Teklad) and water were provided ad libitum. Environmental enrichment was provided. To maintain Tamb after surgery, the rat's cage was equipped with a device that circulated temperature-controlled air into the cage under the Hepa-filter cage top with flow rate 2L/min (Figure 1). The Tamb can be controlled at values ranging from ~17 °C - 35 °C. A thermometer was inserted into the cage to confirm Tamb was tightly regulated. Otherwise, the rat's environment was the same as before surgery in regards to food and water access, circadian light rhythms, etc. All animal housing and experimentation was done in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgical Procedure

Using aseptic techniques as outlined in the NIH Guide for the Care and Use of Laboratory Animals, rats were anesthetized (75 mg/kg ketamine and 15 mg/kg xylazine intraperitoneally). Body weight was measured before surgery to enable accurate calculation of anesthetic dose and to provide a pre-surgical body weight for comparison. Abdominal fur was shaved and the skin was scrubbed with a 10% Proviodine-iodine solution. A ~2 cm midline incision at the lina alba exposed the abdominal cavity and a sterilized radiotelemetry thermoprobe (Data Sciences, #TA-10F40) was surgically implanted to measure Tc (Figure 2). Abdominal muscles were then closed with individual sutures (3.0 silk) and the skin closed with surgical staples. Using stereotaxic approaches, rats were cannulated (23 gauge; 28mm) in their brainstem allowing for microinjection of drugs to modify neurotransmission as part of a separate study. This study would begin after the completion of the recovery period from surgery. After surgery is complete, rats were injected with saline (1ml/150g body weight: intraperitoneal) to replace fluid lost during surgery. Ibuprofen analgesic (0.2mg/ml in water supply) was administered during the surgical recovery period. Non-steroidal anti-inflammatory drugs (NSAIDS) have been shown to be more efficacious for the alleviation of post-surgical reductions in body weight and food and water intake following surgery than buprenorphine (Leon et al., 2005). Adding the analgesic to the water supply would ensure delivery of the drug. In the past, analgesia was added to rat treats for oral administration. This is no longer done because it was difficult to confirm full ingestion of the treat and therefore unnecessary handling of the animal was required to confirm treat consumption, which causes animal stress. Often, the treat was refused by the rats, regardless of the flavor variation in the treat or the NSAID used.

Figure 2: Radiotelemetry Probe. Non-invasively measures Tc from the rat.

Experimental Groups:

Immediately following surgical instrumentation, body weight was measured to enable calculation of body weight lost during surgery. Subjects were then randomly placed in one of five experimental groups where Tamb is maintained at either 21°C, 24°C, 27°C, 30°C, or 33°C following surgery (Table 1). The 27°C group acted as the control because this is the preferred Tamb that unstressed rats select when allowed to choose the Tamb (Brown et al., 2011).

Table 1. Experimental Design. 27°C is the control group as it is the rat's preferred Tamb according to previous findings. UCT: Upper Critical Temperature. LCT: Lower Critical Temperature in relation to the thermoneutral zone of the rats.

Tamb During Surgical Recovery		**N =**	**Experimental Question**
21°C	Typical Room Temp / Cold Stress	5	Does housing rats at normal room temperature cause a cold stress, which hinders surgical recovery?
24°C	Mild Cold Stress	5	Does housing rats at their LCT alter recovery from surgery?
27°C	Preferred Ambient Temp (Control)	5	Does housing rats at their preferred Tamb affect recovery? Other groups will be compared to this control.
30°C	At UCT	5	Does housing rats at the UCT alter recovery from surgery?
33°C	Above UCT / Mild warm stress	5	Does a mild warm stress facilitate or hinder surgical recovery?

Data Collection

The weight of the rat as well as food and water intake was collected daily throughout experimentation. Measurements would occur daily at ~8AM to minimize variation in when experimental parameters were sampled and to minimize the handling stress on the animals. Core temperatures and motor activity were gathered every 5 minutes using the radiotelemetry probe during the recovery period. This was an automated process because the radiotelemetry probes allow non-invasive measurement of physiologic variables such as Tc, thereby eliminating the effect of handling stress on these variables. The recovery period continued until all three of the following conditions were met: 1) at least one week had passed since surgical instrumentation 2) body weight had returned to pre-surgical levels, 3) a clear circadian rhythm of Tc was reestablished. Rats that did not meet these criteria were not used in analysis. These data were organized into smaller bins (~30 minutes) and displayed on a 24-hr axis so circadian rhythms could be evaluated. The time to return to a normal core temperature following surgery (defined as 36.5°C) was evaluated as well as the time to return to normal circadian rhythm.

Data Analysis

The data was analyzed to determine the effect of Tamb on both the time to recover to normal Tc and body weight as well as the time to recover a stable circadian rhythm for Tc. Daily food and water intake were also measured to note any significant differences from previous days and between the Tamb temperature groups. Given that the sample sizes of the Tamb groups were small (n=5), a Shapiro Wilkes test was performed to determine the distribution of the data. Groups that had a normal distribution ($p > 0.05$) would be tested with a One Way ANOVA with the post hoc Tukey's procedure. Data that was skewed ($p \leq 0.05$) was tested using the Kruskal Wallis test, followed by the Nemenyi post hoc test.

Immediately following surgery the initial Tc recordings began. After surgery, the rats exhibited lower core temperature due to loss of body heat, which results from the effect of anesthesia (Diaz & Becker, 2010). It was expected that the Tc would return to normal in the hours after surgery and then begin fluctuating within the normal range of 36.5°C and 38.5°C in the days after surgery under circadian cycle influence. To determine the effect of Tamb on recovery to a normal Tc, the lowest value of the physiological Tc range (36.5°C) was used as the marker point. This was used because once the animal's Tc rises above 36.5°C it will not typically decrease below this unless an outside physiological stress causes the decrease (Gordon, 1993). Each 5-minute interval was measured as a count or a single data point (1 count = 5 minutes). These values were added together to then analyze for differences between the Tamb groups.

Circadian rhythm refers to the normal physiologic fluctuations in various factors, such as Tc, that occur during the daylight to nighttime transitions. Since surgeries were done at varying times throughout the day all Tc data was aligned at 19:00 on the day of surgery, which is the start of the first dark phase of the photoperiod. This enabled circadian rhythm analysis between animals because after that initial photoperiod change they were all on the same light:dark cycling; the same as what they experienced pre-surgery in the vivarium. Two different methods were used to determine the recovery of circadian rhythm. Initially, the mean values from 10:00 AM – 4:00 PM versus 10:00 PM – 4:00 AM of each rat were compared to determine if a significant difference between day and night existed, and therefore, indicate establishment of normal circadian rhythms. This was used as the established time period between active and sleep periods of the rodent. Time periods outside of the 10-4 window were ignored when determining circadian rhythms because the rat is typically in transition from light to dark or dark to light photoperiods and therefore their Tc is unstable. Similar to the Tc recovery, every 5 minutes up to first day or night difference at which circadian rhythm was established was counted as one data point. This was compared among the groups to determine any differences.

The second method to analyze circadian rhythm was a spectral density. Spectral densities are used on time series to determine where there are periodicities, or rather in this study, rhythms within the data. The analysis is a process of representing any periodic function as a harmonic series. This means that the time series can be written as a linear combination orthogonal trigonometric function (such as sine and cosine functions). Spectral analysis uses Fournier transform techniques to uncover a few recurring cycles of different lengths in the time series of interest. This describes a signal as a function of frequency, so when defined as a wave, the unit becomes watts per hertz (W/htz). The bandwidth describes the frequency position of a series as the Fourier transform bin size, which helps identify the smallest frequency for the series. The value of the highest peak indicates periodicity within the data series. The rhythms were represented by a frequency between 0 and 0.5 (*f*). This value was divided with 1 (1/*f*) to produce a whole number for comparison. Rhythms that were displayed early in the spectrum density analysis were a higher frequency, showing circadian rhythm had established within the first few days after returning to normal Tc. Periodicities with lower frequencies would show a delay in the stability of a circadian rhythm. In this analysis, the variation or noise in the data was smoothed by averaging every 2 hours of Tc data. The spectral density would mistake the noise as a periodicity, which could be misinterpreted.

The effect of Tamb on rat survival following surgery was also analyzed. The number of rats that survived at least 1 week of recovery at each Tamb was compared to the total number of rats in each Tamb group to determine survival rate. Rats that did not survive the surgery were excluded from this analysis since those rats never entered the Tamb cages and thereby never began the recovery process.

Results

MI388 is an example of a rat from the 27°C group that returned to a normal Tc (36.5°C -38.5°C) and displayed circadian rhythm at about 24 hours or a day after the surgery (Figure 3). Note that after returning to 36.5°C, the Tc does not go below that degree throughout the week. Each black box represents a dark photoperiod (nighttime), while the unmarked areas represent the light photoperiod (daytime). The Tc rises during the night period and decreases during the day, illustrating the circadian rhythm.

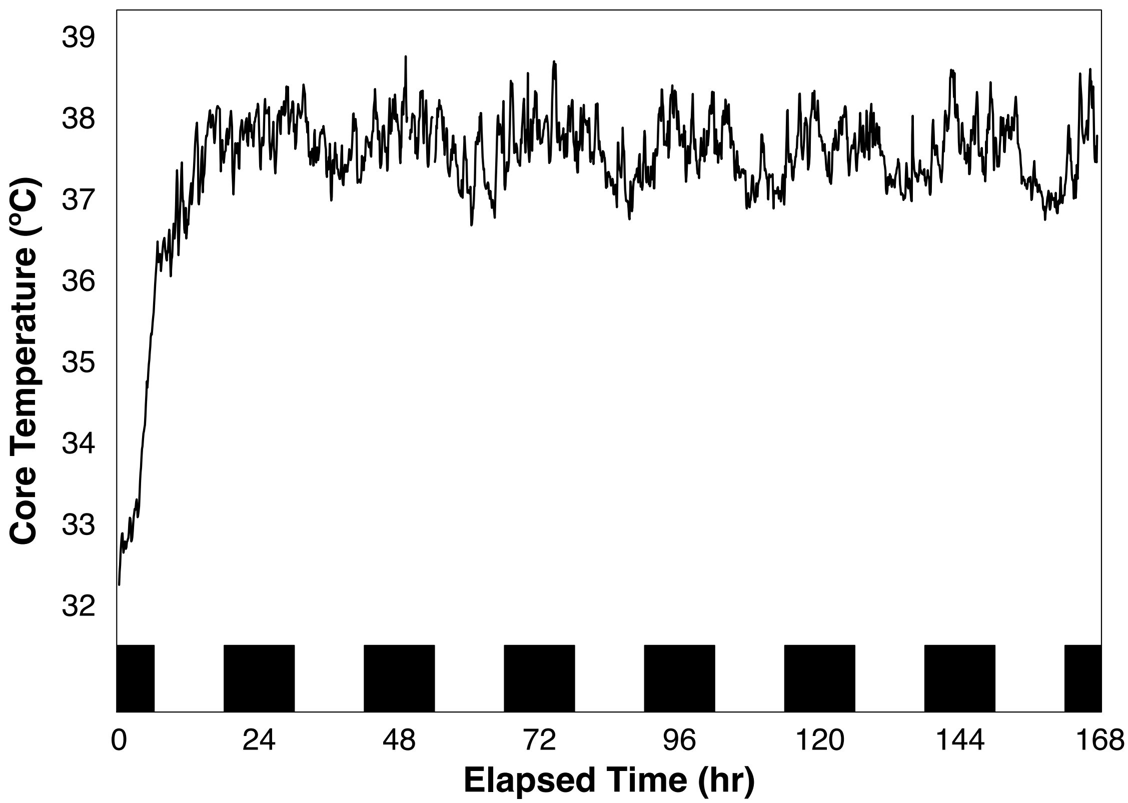

Figure 3. The Typical Tc Recovery Response. MI388 is an example of a rat housed at Tamb 27°C that returned to 36.5°C within a day and displayed circadian rhythm a day after the surgery. This rat returned to normal circadian rhythm after the second photoperiod.

Core Temperature Recovery

Figure 4 illustrates a general trend where the rats housed at Tamb 33°C return to a normal Tc faster than the other groups whereas the 21°C group takes longer. However, there are no significant differences between the Tamb groups. Small circles found in all of the boxplots represent outliers in the data.

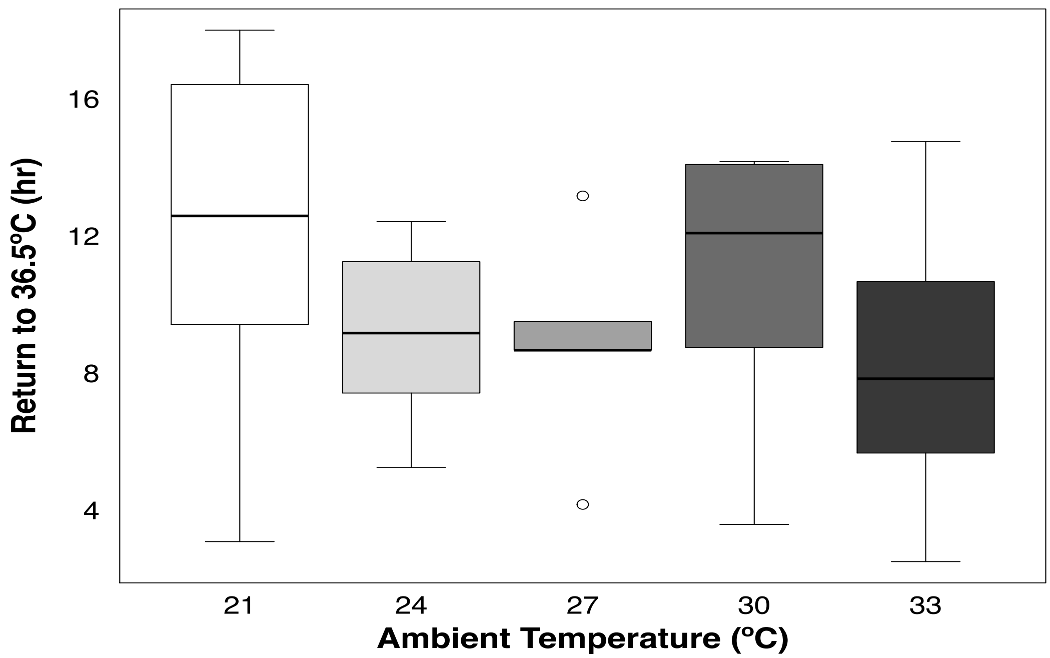

Figure 4. Return of Tc Following Surgical Procedure. Tamb 33°C returned to a normal Tc faster than the other Tamb groups versus the Tamb 21°C which took longer. (Kruskal-Wallis, p=0.6545)

Circadian rhythm

Figure 5 is an average of all the Tc from all the Tamb groups. The data was put into 2 hour bins to display the rhythms. The 21°C group appears to have a slight delay in returning to circadian rhythm whereas all groups exhibit a circadian rhythm similar to each other after 120 hours (5 days).

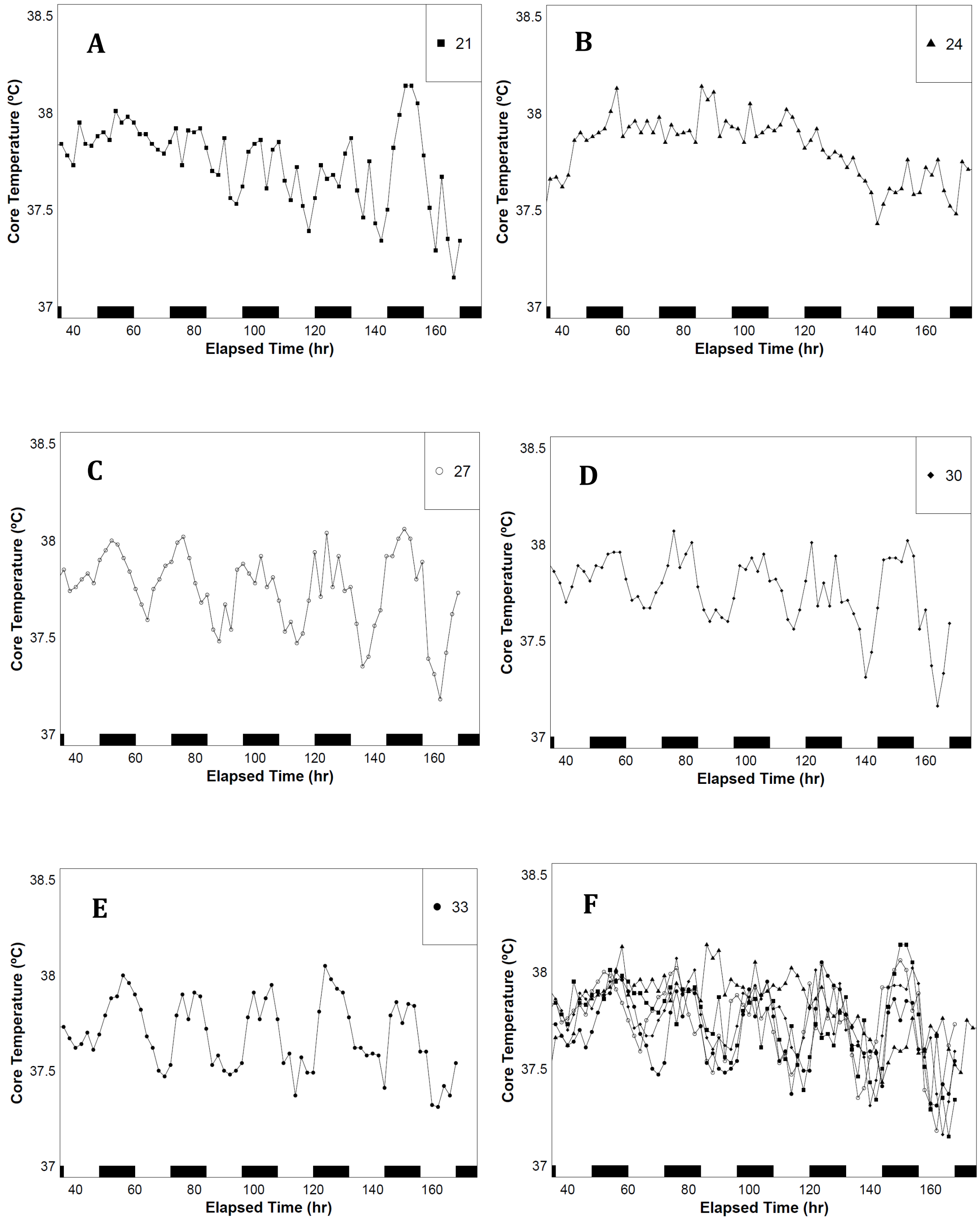

Figure 5. Averages of all the Tamb Circadian Rhythms in the Recovery Period. The data presented here is of a 2 hour interval average from hour 40 (~1.75 days) to hour 168 (7 days) after surgery. A. 21°C. B. 24°C. C. 27°C. D. 30°C. E. 33°C F. Data from all Tamb groups.

Figure 6 illustrates the time it took for rats in each group to re-establish their Tc circadian rhythms using the "10-4" method of data analysis. Although there are no significant differences observed, the warmer Tamb groups, and the control group (27°C), seem to establish circadian rhythm faster than the colder Tamb. Again, there is a delay in the establishment of circadian rhythm in the 21°C group. Without the outliers present (one in each of the 21°C, 27°C and 33°C groups), there is a significant difference between 21°C and 27°C, and again between 21°C and 33°C (Fig. 6).

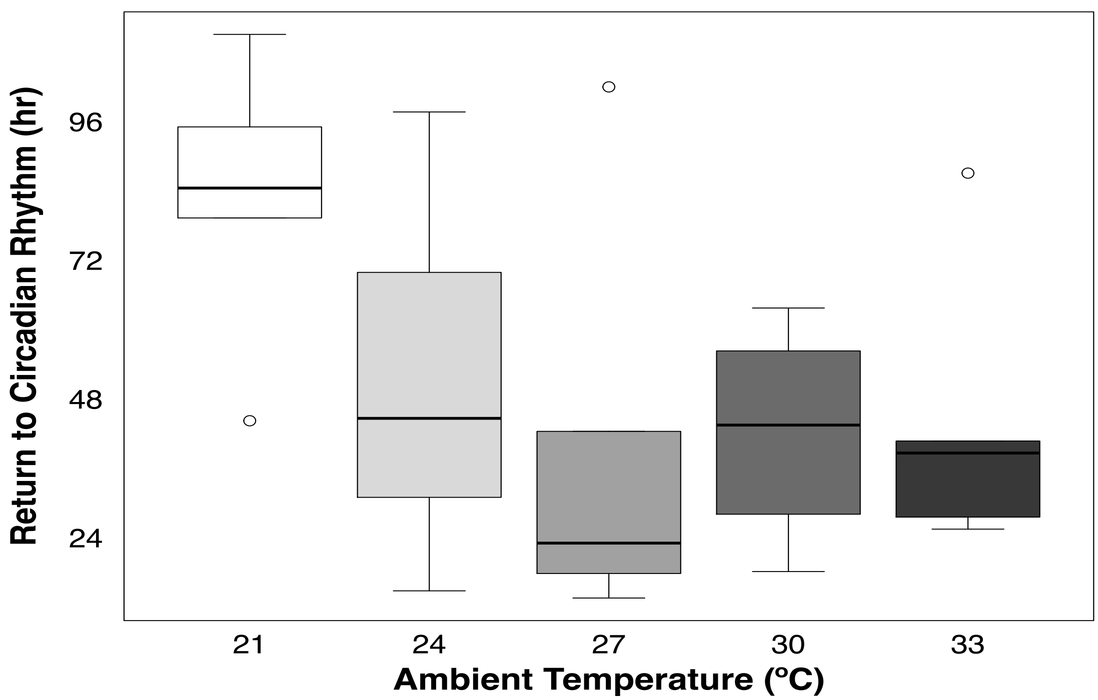

Figure 6. The 10-4 Period Comparison of the Establishment of Circadian Rhythm. The warmer Tamb groups, and the control group (27°C), stabilize circadian rhythm faster than the colder Tamb groups (ANOVA, p=0.1367).

The second method to determine circadian Tc rhythm recovery used spectral density to express the data as a frequency. Figure 7A shows MI388 (Figure 3) with smoothing applied, clearly illustrating the circadian rhythm. Figure 7B show the spectral density with the earliest periodicity before 0.1. The larger the percentage, the sooner the circadian rhythm was established. Figure 8 displays the intergroup comparisons of all the rats. The results there are similar to Figure 6 which used the "10-4" method to analyze circadian rhythm recovery. The 21°C group had the smallest percentage, meaning the periodicity was not detected until later in the week.

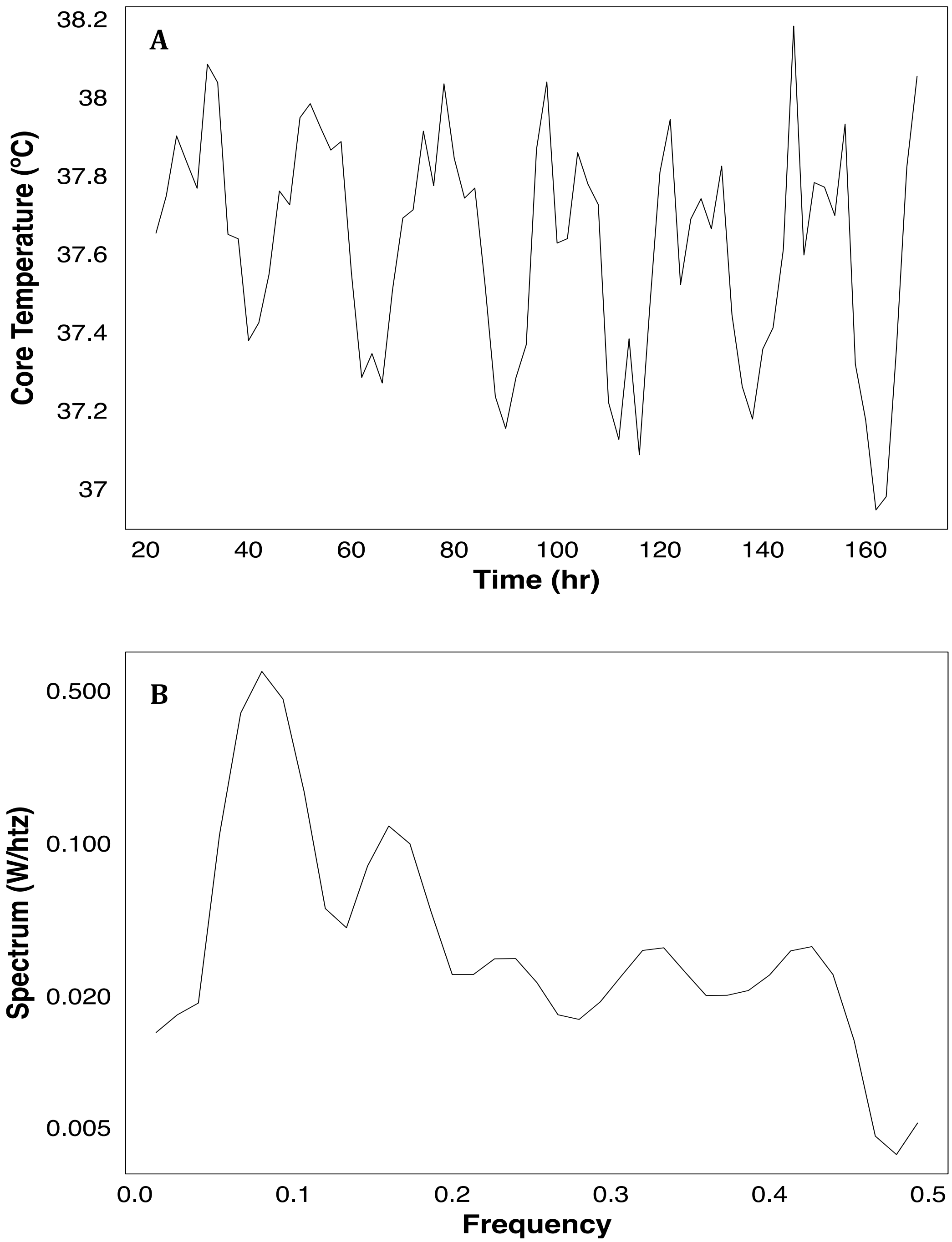

Figure 7. A. MI388 2 Hour Average. This illustrates the circadian rhythm and reduces the variation. B. Spectral Density of MI388. The highest spectrum dictates early periodicity of circadian rhythm establishment.

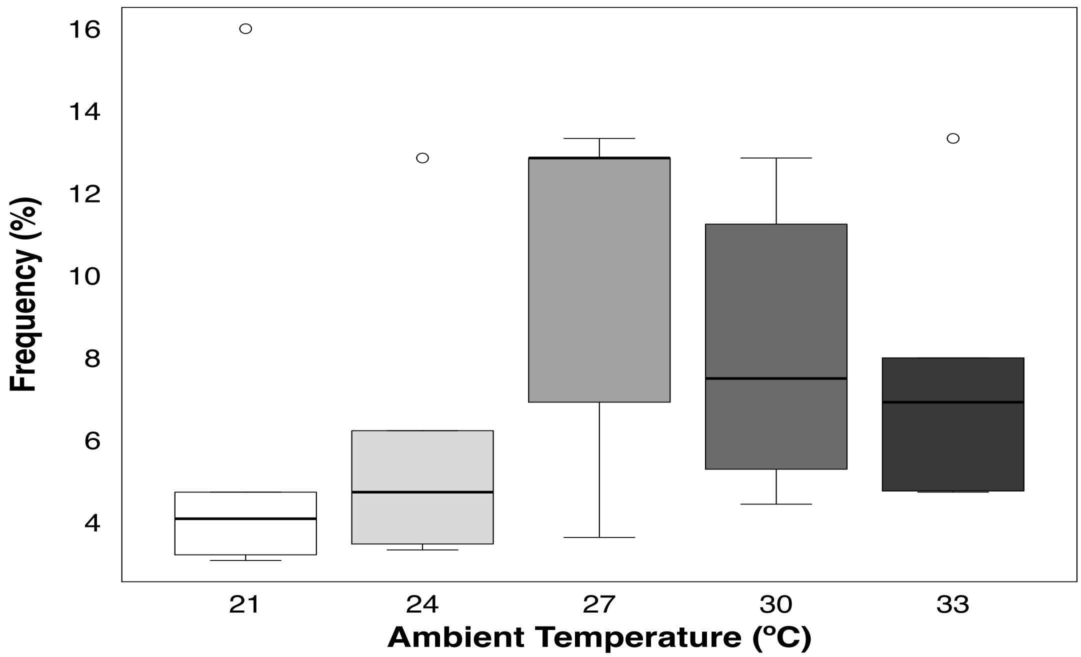

Figure 8. Spectral Density Comparison of Establishing Circadian Rhythm. The 21°C group has a delay in the return of circadian rhythm while the 27°C group established circadian Tc rhythms the soonest. (Kruskal-Wallis, p=0.3227)

Body Weight

The percent changes in rat's body weight are illustrated in Figure 9. There was no significant difference between the groups despite a general trend that the 21°C group seemed to lose more weight and recover slower than the other groups while the 27°C group seemed to recover the quickest. If the change in weight from one individual day to the next is compared across the groups, then the rats housed at 21°C did lose significantly more weight on day one when compared to rats housed at 33°C (Figure 10 and 12). When total weight lost over the entire week is analyzed (Figure 11) the 21°C group seemed to lose the most weight but it was not significantly different than the other groups.

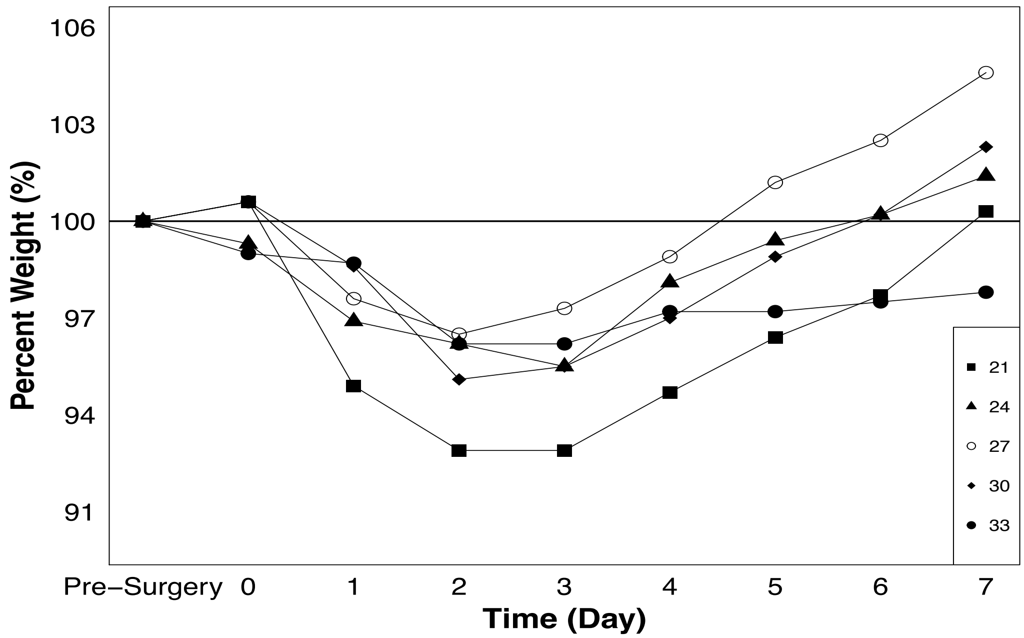

Figure 9. The Percent of Original Body Weight Lost in Each Tamb Group During the Recovery Week. Data presented as mean. N=5 in each group.

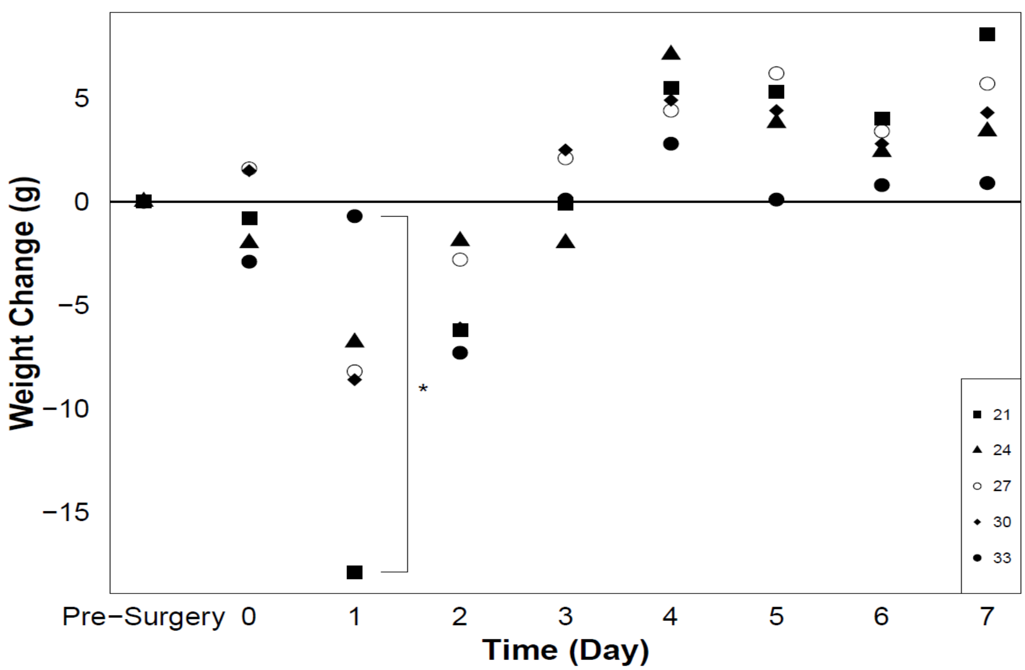

Figure 10. The Average Weight Change Per Day Following Surgery. 21°C lost the most weight on Day 1 but regained that weight back around Day 4. *Significant difference between 21°C and 33°C on Day 1 (Tukey post hoc, p=0.006).

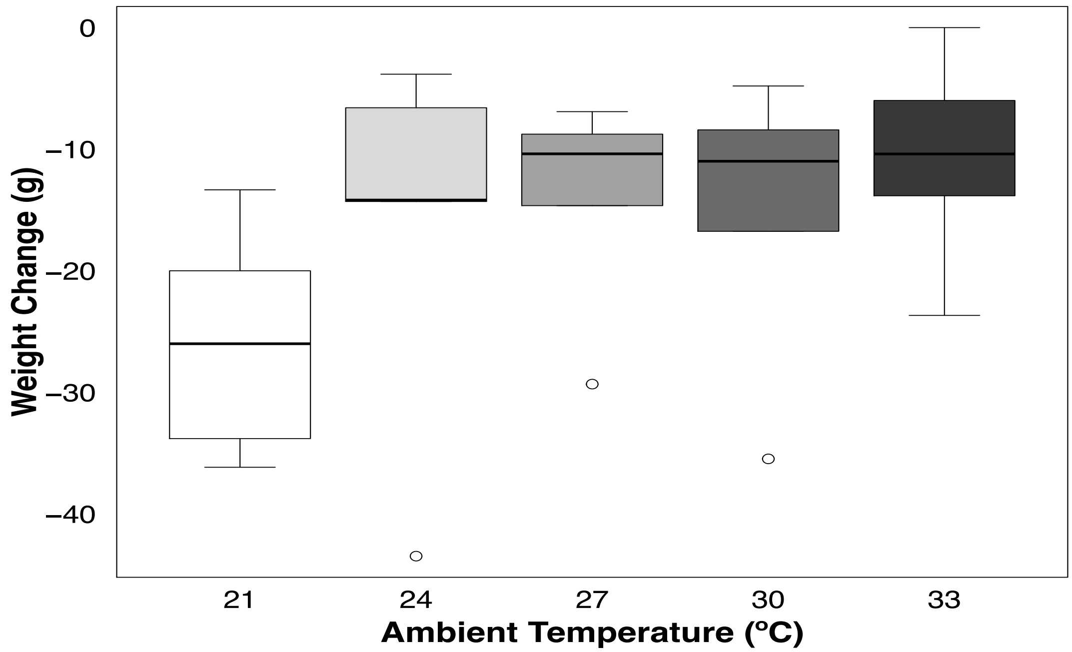

Figure 11. The Total Amount of Weight Lost Following the Surgery. The 21°C group lost the most weight during the week but it was not significant in comparison to the other groups (Kruskal-Wallis, p=0.292).

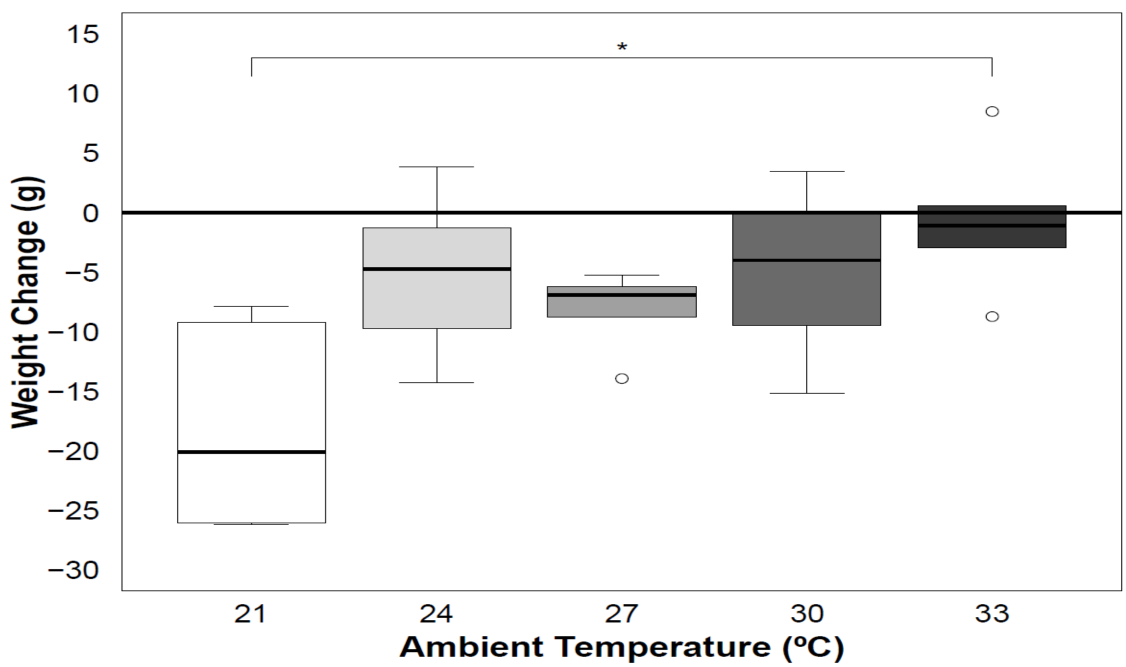

Figure 12. The amount of weight lost between Day 0 and Day 1. There was a significant difference between 21°C and 33°C. *Indicates significant difference between groups (Tukey post hoc, p=0.006).

Finally, the body weight recovery between Days 1, 3 and 7 was analyzed within and between groups. Day 1 followed the surgery. Day 3 was the period in which either the weights had begun to stabilize or show minimal change. Day 7 was the final measurement when most rats had returned to their pre-surgical weight. There were no differences of the daily weight change between the Tamb groups, but there were differences within the select Tamb groups on Day 1 and 7. Specifically, in the 21°C and 27°C groups the weight change on Day 1 was significantly different than the weight change on Day 7 (Figure 13).

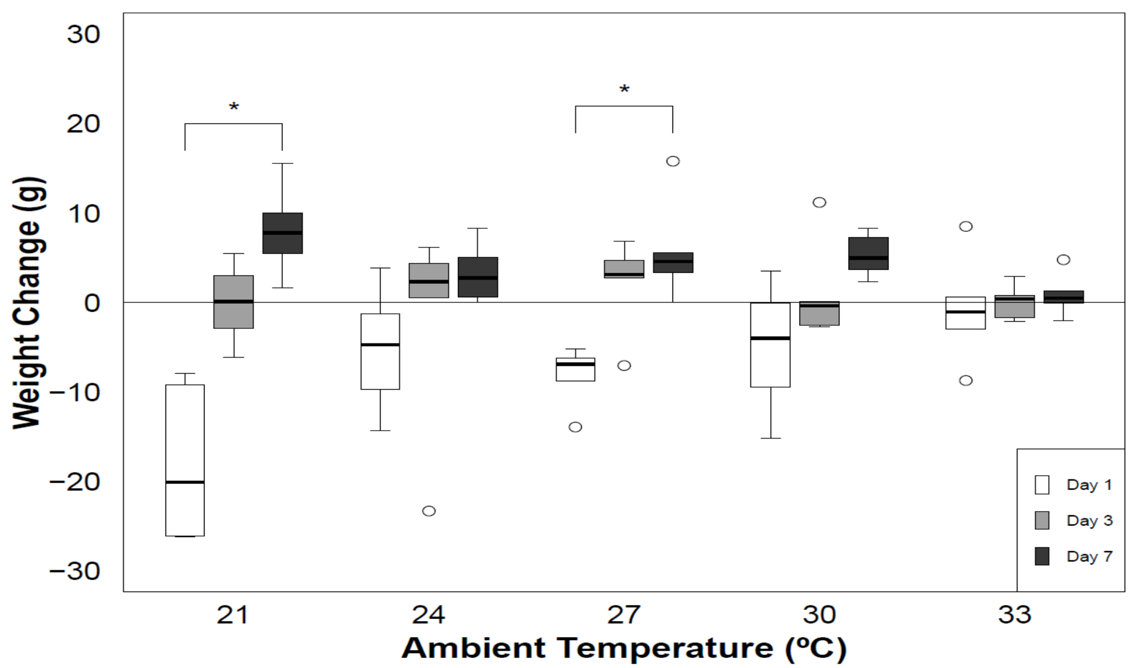

Figure 13. The Amount of Weight Change of Days 1, 3, and 7 Between the Tamb Groups. There were no differences between the groups, only between days on individual groups. The differences were between Day 1 and 7 of Tamb 21°C and Day 1 and 7 of Tamb 27°C. *Indicates significant difference between days (Nemenyi post hoc, 21°C p=0.023; 27°C p=0.02).

Food and Water Consumption

Food and water were measured daily following the surgery. Figure 14A represents the averages of food consumed for each Tamb group. Between Day 1 and Day 2, the 24°C group ate the most food which then stabilized later in the week. In contrast, the warmer groups ate less food, especially later in the week. In the total amount of food consumed during the week (Figure 14B), there were no significant differences but it seems the colder groups ate more food versus the warmer Tambs. Within the 21°C group (Figure 15), there was a significant difference between Day 1 and Day 7 food consumption, but there were no other differences observed in the other Tambs. There were no differences between the Tamb groups.

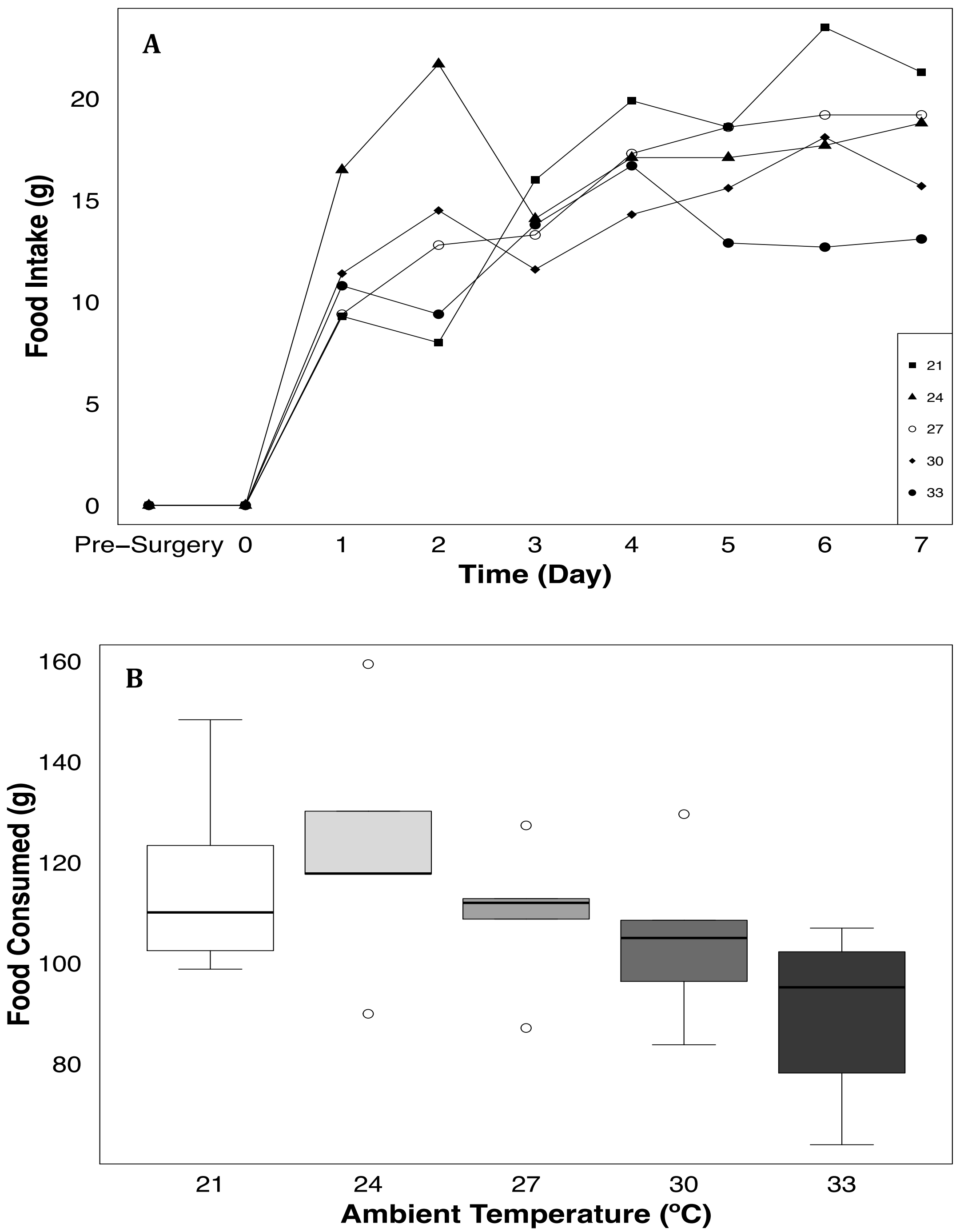

Figure 14. A. The Averages of the Food Intake Following Surgery Over 7 Days. B. The total amount of food consumed over the week (Kruskal-Wallis, p=0.098).

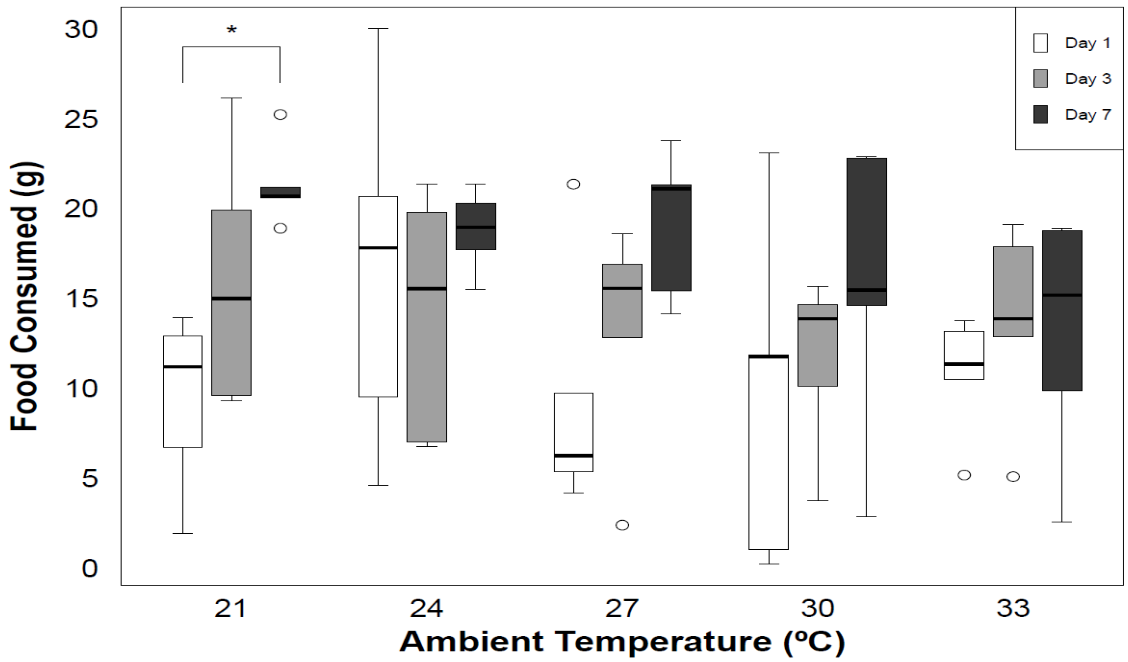

Figure 15. The amount of food consumed between Day 1, 3, and 7. The only difference observed was between Day 1 and Day 7 of the 21°C group. *Indicates significant difference between groups (Tukey post hoc, p=0.02).

Water consumption shares a similar trend between the groups. The amount of water consumed increases throughout the week (Figure 16A). The warmer groups (30°C and 33°C) as well as the 27°C group, all seem to have an increased water consumption in comparison to the colder Tambs despite the lack of significance in the data (Figure 16B). The 27°C group has significant increases in water intake between Day 1 and both Day 3 and Day 7. The rats in the 30°C and 33°C groups had significant increases in water consumption between Days 1 and 7 (Figure 17).

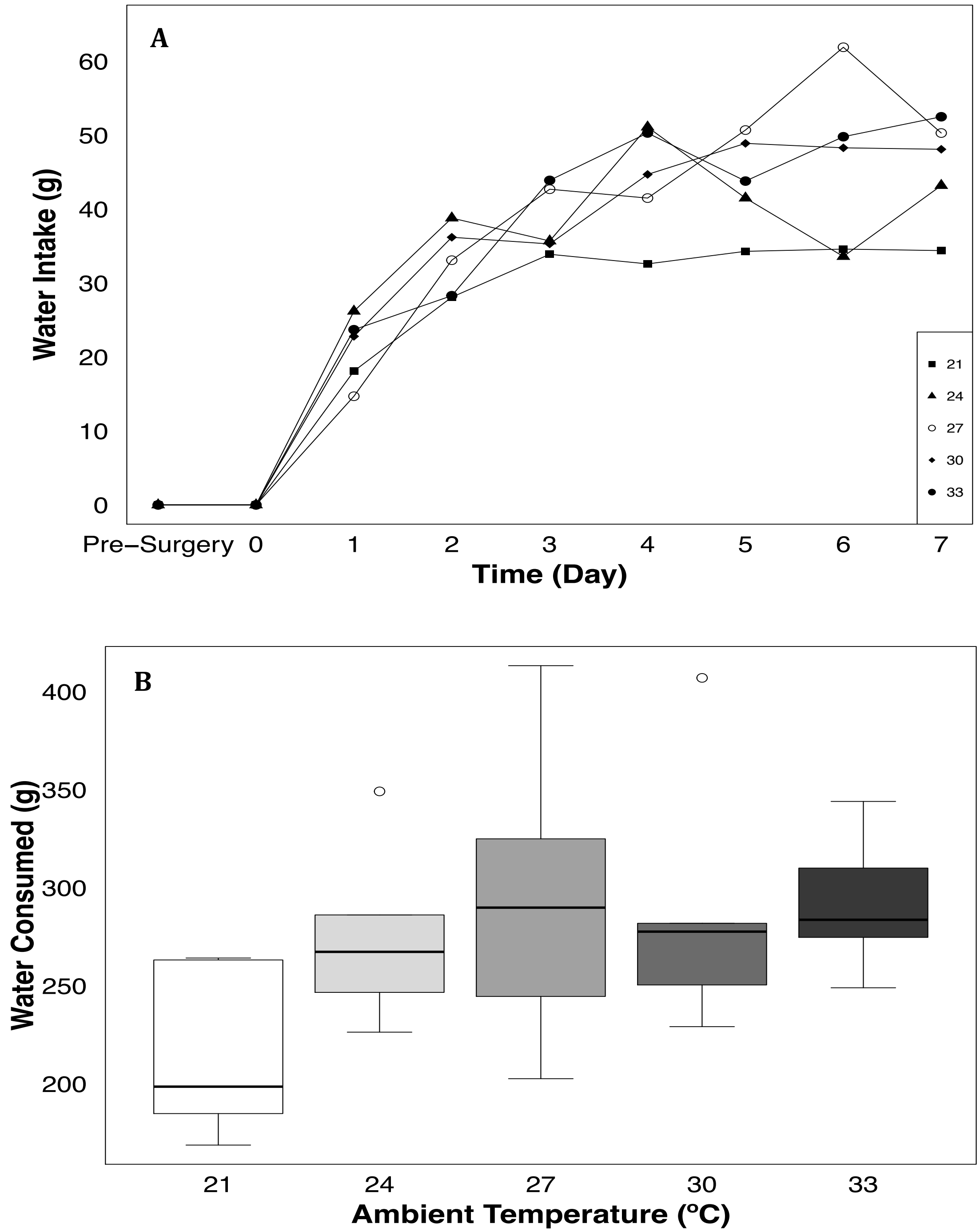

Figure 16. A. The averages of water consumption following surgery over 7 days. B. The total amount of water consumed over the week (Kruskal-Wallis, p=0.168).

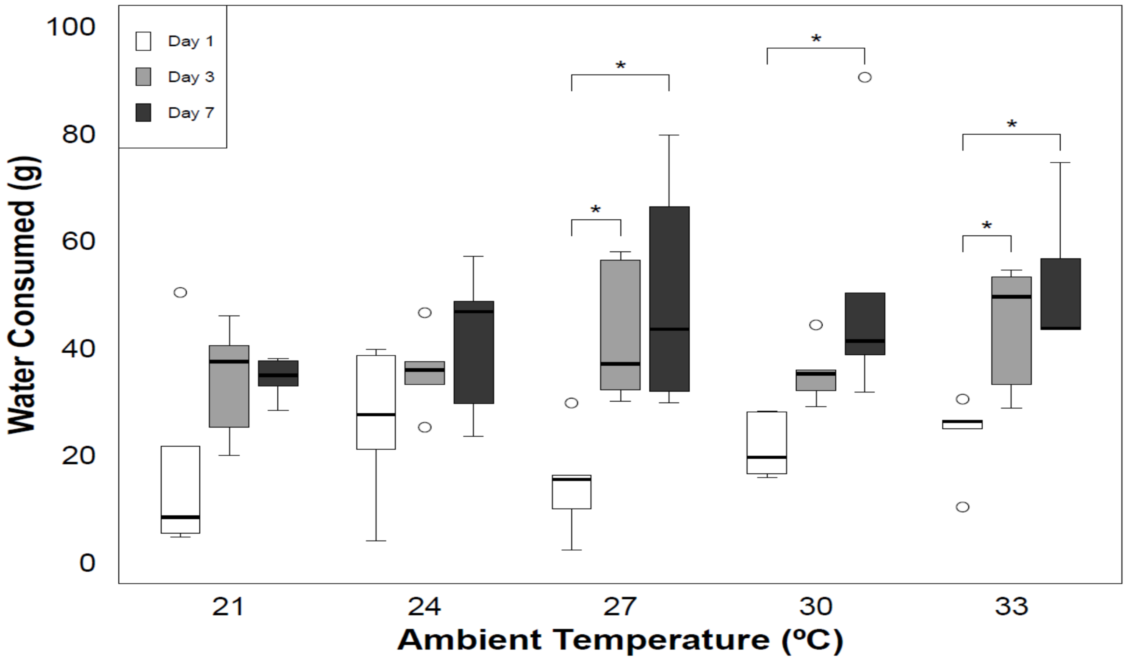

Figure 17. The Amount of Water Consumed Between Day 1, 3, and 7. Differences were not observed between the Tamb groups. The 27°C group had differences between Day 1 and Day 3, and Day 1 and Day 7. The 30°C groups only had differences between Day 1 and Day 7, while 33°C had differences between Day 1 and Day 3, and Day 1 and Day 7. *Indicates significant difference between days (Tukey post hoc, 27°C: p=0.041, 0.011; 30°C, p=0.019; 33°C, p=0.039, 0.004).

Survival

A survival percentage was calculated for each experimental group. The 21°C and 30°C groups have the lowest survival rate with 71.43% whereas the 24°C, 27°C, and 33°C had higher survival rates of 83.33%.

Table 2. The survival percentage of the Tamb groups.

Tamb (C°)	Survived 24 hours	Total surgeries	%
21	5	7	71.43%
24	5	6	83.33%
27	5	6	83.33%
30	5	7	71.43%
33	5	6	83.33%

Discussion

Core Temperature Recovery

An important aspect of recovery from a surgical procedure is the return of Tc to normal. With anesthesia, the blood vessels vasodilate and the hypothalamus is unable to thermoregulate, causing heat to leave the deeper tissues and travel to the skin (Diaz & Becker, 2010). When returning to normal Tc, the hypothalamus of rats again begins to control thermoregulation and stimulates brown adipose tissue to generate heat using non-shivering thermogenesis. In this study, we stated that Tc had returned to normal when the rats Tc had reached 36.5°C because this temperature is at the lower extremity of a normal Tc range for this strain of rat. It is important to note that while recording Tc during recovery the Tc was above 36.5°C throughout the week, supporting the use of 36.5°C as the demarcation point for a return to normal Tc. Interestingly, there were no differences observed between the groups in their Tc return to 36.5°C (Figure 4). Noticeably, the 21°C group has a much wider variation or spread compared to the other Tamb groups. The addition of more animals may reduce this variation and perhaps show significance between the 21°C group and others. It was expected that the rats in the 21°C group would have difficulty returning to their normal Tc because within a colder environment, the effects of anesthesia causing vasodilation are more drastic. Therefore, more blood is moved to the periphery and consequently environmental heat loss is increased. Without the aid of external heating, such as heating air-systems, this thermoregulatory impairment can complicate recovery of a normal Tc (Diaz & Becker, 2010). The use of external heat warmers during recovery is very helpful. Although not significant, this is shown by the 33°C group in Figure 4, having recovered the Tc faster than the rest of the group. With additional animals included in the study, there could be a significant difference seen between 21°C and 33°C groups.

Circadian Rhythm

Establishing a circadian rhythm is essential to showing normal physiologic behavior. Not only is this rhythm seen in Tc, it is also present in physiological processes such as motor activity, corticosterone levels, and liver metabolism. The advantage of having circadian rhythms is being able to anticipate environmental changes and manage our physiological processes to them (Narasimamurthy & Virshup, 2017). Experiments that do not account for disrupted rhythms could inadvertently add another variable that affects the data. The 10-4 periods show differences between the daytime and night time Tc levels and thereby determine the establishment of circadian rhythm. Using this method to analyze the data, the establishment of a circadian rhythm after surgery was mildly delayed in the 21°C group. Although this data was not significantly different, the delay in the 21°C group recovery could be concerning for housing SD rats at room temperatures, which are comfortable to those caring for the rats but lead to alterations in thermoregulatory function following surgery. This suggests the recovery process can take longer than anticipated in these conditions. If the Tc remains unstable, then data gathered from these animals may be misleading. The spectral density method yielded similar results to the 10-4 periods, including a delay in the establishment of circadian rhythm in the 21°C group. It is important to note that both the 10-4 periods and the spectral density methods show there are negative effects when housing animals within 21°C.

In contrast to the 21°C, the warmer groups and those housed at 27°C, establish a circadian rhythm within the first few days of recovery. This is essential to this study because the 27°C Tamb is the preferred Tamb of SD rats (Brown, 2011). Without the one outlier that is present in the 21°C data, the establishment of the circadian rhythm in this group would be significantly delayed when compared to the 27°C and 33°C groups. Therefore, it is essential that the current sample size (n=5/group) be increased. This should elucidate these differences and support raising the Tamb within the first days of recovery around 33°C and then decreasing the Tamb to 27°C. It was interesting to see that there was a mild delay in the establishment of circadian rhythm in the 30°C and 33°C groups, although not as delayed as the 21°C Tamb. Since these Tambs are at and above the upper range of the preferred Tamb, these warmer temperatures are probably causing a heat stress later in the recovery week. This likely increases their evaporative water loss and results in an increase in their water intake seen in Figures 16 and 17 (Gordon, 1993). Alternatively, the delay in establishment of a normal circadian rhythm could have been caused by a period of acclimation to the warmer temperatures. However, further research is needed to determine if acclimation does have an influence on circadian rhythms in the warmer Tamb groups.

Weight, Food, and Water Measurements

Weight changes following surgery are often measured to ensure the rat is healthy. This is considered to be more beneficial information because normal growth rates following surgery can be hindered by numerous factors such as external stressors, postoperative pain, and pain management drugs (Brennan et al., 2009). These stressors influence the return of weight to normal pre-surgical levels. Only a few rats did not regain their original body weight by Day 7. The few rats that failed to regain their pre-surgical body weight did not affect the overall data trends. The cold stress from housing at 21°C caused a greater decrease in body weight compared to other groups. This was evidenced by the difference between Day 1 weight loss between 21°C and 33°C (Figure 10 & 12). This was likely due to an increase in metabolic rate and subsequent loss in body weight following the surgical procedure. Metabolic rate is known to increase when animals are maintained at Tamb below their TNZ. In an experiment conducted by Wang et al. (2010), SD rats housed in a cold stress environment showed decreasing body weight throughout the experiment, but in contrast, there was an increase in brown adipose tissue, the tissue essential for non-shivering thermogenesis. Their findings suggest the cold stress increased metabolic rate, leading to a dependence on non-shivering thermogenesis to produce heat. Increasing food consumption would be required to meet the now higher metabolic demands on the animal to both recover from surgery and maintain an elevated metabolic rate. Rats housed at 21°C seemed to have increased food consumption earlier in the recovery week, which supports this conclusion (Figure 14A). However, once the animal adapted to the colder environment the food consumption difference between the 21°C group and the others were negligible. They did lose the most weight on Day 1 but they regained that weight quickly, which reduced the significance of the total weight lost for the week. Furthermore, rats housed at 33°C quickly recovered their Tc (Figure 4) and therefore did not require excessive food consumption to support metabolic heat production early in the week (Figure 14). There is also more variation in the weight loss of Day 1 in the 21°C group, whereas the 33°C group is more stabilized, showing similar weight loss. Later in the week those rats seemed to not need to consume as much food as the other groups. However, more animals are needed in the study to confirm the conclusions.

Measuring food and water intake can be helpful in determining physiological changes in lab animals. For example, in a study conducted by Sharp et al. (2003) analyzing the effects of different analgesics following a major abdominal surgery, the rat's water consumption returned to normal within 5 days; however, food consumption was dependent on the analgesic that was used during recovery. The recovery of normal pre-surgical food intake varied between 5 and 12 days. Another study investigated the effects of chronic stress and found evidence of a relationship between food intake and body weight changes. With the increased duration and intensity of the stressor, food intake and body weight gain would decrease from chronic restraint and immobilization stressors (Marti et al., 1993).

Water intake is another measurement commonly used to identify health issues in laboratory animals. Rats lose a large amount of fluid during a surgery from the anesthesia's effect on bladder control and evaporative water loss from exposing body cavities during surgery. This is why saline is given following the surgery to replace the fluid lost (Institute for Laboratory Animal Research, 2011). Furthermore, animals housed in warmer Tamb environments were expected to consume more water to offset evaporative water lost to the warmer environment. In fact, the warmer Tamb groups and the control group (27°C) demonstrated a mild increase in water consumption compared to cooler Tamb groups (Figure 16). This was especially true as the week progressed. This is supported by the significant increase in water consumption between both Days 1 and 3 and 1 and 7 in the 27ºC group. Similar trends of increasing water consumption as the recovery week progressed can be seen in the 30ºC and the 33ºC groups as well. These trends are not as apparent in the cooler Tamb groups. This supports the idea that when housed at warmer Tamb, the rats consume more water to replace the extra fluid lost via evaporation from the skin and the respiratory tract to the environment.

Survival

Table 2 illustrates the survival rate of the rats that were included in the study. The table shows that 21°C and 30°C groups had the lowest survival rate (71.43%) and 24°C, 27°C, and 33°C had the highest (83.33%). With further experiments, we expect the percentages to change within some of these groups. From the previous data that was gathered prior to the decontamination of the vivarium, 21°C showed a much lower percentage, less than 50%. With further experiments, we also believe that the current 21°C group will decrease. Most likely, the cold stress environment increases metabolic activity required to produce the required body heat to thermoregulate. Caloric intake would have to increase to support this now increased metabolism. This is supported by the great loss of body weight, and an increased food intake in those rats. It is interesting to see that the 33°C survival rate is high, given that this Tamb is considered a mild heat stress. If the recovery period was extended from 1 week to 2 weeks, there could be destabilization of circadian rhythm, indicating a stressed rat. This may decrease survivability. Previous attempts of housing rats in 36°C following surgery was problematic and discontinued because of the low survival rate from the increased heat rate. With an increased recovery period duration, we could see a decrease in the 33°C survival rate. Further experiments are needed to determine the effects of Tamb on survival rate.

Conclusion

The goal of this experiment was to investigate the ideal Tamb in which SD rats could recover following a major surgical procedure. The return to normal Tc and the reestablishment of circadian rhythm were the main criteria for showing normal physiologic characteristics as well as recovery of body weight, and normalization of food and water consumption. The 33°C group returned to normal Tc the fastest while 27°C established circadian rhythm within the first day after surgery. Food and water consumption were also likely affected by Tamb after surgery. Rats housed in a cold Tamb required more food intake to meet increase metabolic demands to maintain body heat early in the week. After acclimation however, these trends dissipated. The alterations in food consumption likely influenced the recovery of body weight to pre-surgical levels in that the 21ºC group seemed to lose the most weight immediately after surgery but recovered that weight quickly. Also, rats housed at 27ºC and above seemed to require more fluid intake throughout the recovery week. This was likely done to offset evaporative water loss at higher Tamb.

These data suggest the use of mild external heating will facilitate surgical recovery in SD rats if that elevation in Tamb is limited to only a few days. Once the Tc has returned to a normal level, body weight has recovered, and feeding and drinking behaviors have been re-established, then the Tamb should be reduced to ~27°C to help establish and maintain a circadian rhythm. Housing animals at a Tamb that is comfortable to research staff (21°C) can be harmful. These data suggest housing at that Tamb has a negative effect on surgical recovery. There is a delayed return to normal Tc and establishment of circadian rhythm when SD rats are housed at 21°C. The delayed establishment of circadian rhythm, along with the weight loss, could confound data interpretation from these animals.

An important aspect to note in this study is the small sample size (n=5/group). With further experiments, an increased sample size should help elucidate significant differences between groups. There are many outliers affecting the data of the 21°C group in particular. When removed, the data show significant differences between the Tamb groups. There are trends seen within the figures that illustrate what was hypothesized. Further research is needed to fully determine the appropriate housing during surgical recovery.

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